

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

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Application No. Unassigned

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For: POLYPEPTIDES THAT BIND HIV
gp120 AND RELATED NUCLEIC
ACIDS, ANTIBODIES,
COMPOSITIONS, AND METHODS OF
USE

Art Unit: Unassigned

Examiner: Unassigned

10/084,813

PENDING CLAIMS AFTER ENTRY OF PRELIMINARY AMENDMENT

I 1. A polypeptide comprising the amino acid sequence YDIXYYXXE,
wherein X is any synthetic or naturally occurring amino acid residue, such that the
polypeptide binds HIV gp120 under physiological conditions, and wherein said
polypeptide comprises less than about 100 contiguous amino acids that are identical to or
substantially identical to the amino acid sequence of the human CCR5 chemokine
receptor. CCR5

2. The polypeptide of claim 1, which comprises less than about 50 contiguous
amino acids that are identical to or substantially identical to the amino acid sequence of
the human CCR5 chemokine receptor. I/10,12,13,14,18
2-7/9-14,17,18
8/9,11,13,14,17,18
15/9-13,17,18
16/9-14,17,18
19-24/9-14,17,18

3. The polypeptide of claim 2, which comprises less than about 25 contiguous
amino acids that are identical to or substantially identical to the amino acid sequence of
the human CCR5 chemokine receptor.

I - CCR5
2 - CXCR4
3 - STRL3
4 - CCR5'
5 - CXCR4'
6 - STRL3'
7 - CD4

8 - CCR5 N.A.
9 - M.M. Ab USING 1
10 - M.M. Ab USING 8
11 - M.F.N.H. USING 1
12 - " " 8
13 - " " Ab/DI
B1

14 - M.M. Ab TO GP120
15 - GP120
16 - GP120-Ab
17 - M.M. Ab USING 1
18 - " " " " Ab/DI
19 - M.M. Ab CXCR4 N.A.
20 - STRL3 N.A.
21 - CCR5' N.A.
22 - CXCR4' N.A.

23. STRL3' NA
24. CD4 NA

4. The polypeptide of claim 3, which comprises less than about 13 amino acids that are identical to or substantially identical to the amino acid sequence of the human CCR5 chemokine receptor.

5. The polypeptide of claim 4, which consists essentially of [?]YDIXYYXXE.

6. The polypeptide of claim 1, which comprises the amino acid sequence YDIN*YYT*S*E, wherein N* is asparaginyI or a synthetic or naturally occurring substitute therefor, T* is threoninyI or a synthetic or naturally occurring substitute therefor, and S* is serinyI or a synthetic or naturally occurring substitute therefor.

7. The polypeptide of claim 6, wherein N* is asparaginyI, T* is threoninyI, and S* is serinyI.

8. The polypeptide of claim 1, comprising the amino acid sequence M*D*YQ*V*S*SP*IYDIN*YYT*S*E, wherein each letter indicates the standard amino acid residue designated by that letter, and a letter followed directly by an * indicates that any synthetic or naturally occurring amino acid can occupy that position.

9. The polypeptide of claim 8, wherein said letter followed directly by an * indicates the amino acid residue represented by the letter or a synthetic or naturally occurring conservative or neutral amino acid substitution therefor.

10. The polypeptide of claim 9, wherein said amino acid sequence is MDYQVSSPIYDINYYTSE.

11. A polypeptide comprising the amino acid sequence XEXIXIYXXXNYXXX, wherein X is any synthetic or naturally occurring amino acid, such that the polypeptide binds HIV gp120 under physiological conditions, and wherein said polypeptide less than about 100 contiguous amino acids that are identical to or

substantially identical to the amino acid sequence of the human CXCR4 chemokine receptor.

12. The polypeptide of claim 11, which comprises less than about 50 contiguous amino acids that are identical to or substantially identical to the amino acid sequence of the human CXCR4 chemokine receptor.

13. The polypeptide of claim 11, which comprises less than 25 contiguous amino acids that are identical to or substantially identical to the amino acid sequence of the human CXCR4 chemokine receptor.

14. The polypeptide of claim 13, which [?]consists essentially of EXIXIYXXXNY.

15. The polypeptide of claim 11, which comprises the amino acid sequence M*EG*IS*IYT*S*D*NYT*E*E*, wherein each letter indicates the standard amino acid residue designated by that letter, and each letter followed directly by an * indicates the amino acid residue represented by the letter or a synthetic or naturally occurring conservative or neutral amino acid substitution therefor.

16. The polypeptide of claim 15, wherein said amino acid sequence M*EG*IS*IYT*S*D*NYT*E*E* is M*EGISIYTSDNYT*E*E*.

III 17. A polypeptide comprising the amino acid sequence EHQAFLQFS, such ^{STRL33} that the polypeptide binds with HIV gp120 under physiological conditions and wherein said polypeptide comprises less than about 100 contiguous amino acids that are identical to or substantially identical to the amino acid sequence of the human STRL33 chemokine receptor.

18. The polypeptide of claim 17, which comprises less than about 50 contiguous amino acid that are identical to or substantially identical to the amino acid sequence of the human STRL33 chemokine receptor.

19. The polypeptide of claim 18, which comprises less than about 25 contiguous amino acids that are identical to or substantially identical to the amino acid sequence of the human STRL33 chemokine receptor.

20. The polypeptide of claim 19, which consists essentially of the sequence EHQAFLQFS.

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IV
21. A polypeptide comprising at least a portion or all of an amino acid sequence selected from the group consisting of LPPLYSLVFIFGFVGNML, QWDFGNTMCQLLTGLYFIGFFS, SQYQFWKNFQTLKIVILG, APYNIVLLLNTFQEFGNCS, and YAFVGEKFRNYLLVFFQK, and, optionally, substituted with up to 6 conservative or neutral amino acid substitutions, wherein the polypeptide binds with HIV gp120 under physiological conditions and comprises less than about 100 amino acid residues that are identical to or substantially identical to the amino acid sequence of the human CCR5 chemokine receptor.

IV
22. A polypeptide comprising at least a portion or all of an amino acid sequence selected from the group consisting of LLLTIPDFIFANVSEADD (165-182), VVFQFQHIMVGLILPGIV (197-214), and IDSFILLEIHKQGCEFEN (261-278), and, optionally, substituted with up to 6 conservative or neutral amino acid substitutions, wherein the polypeptide binds with HIV gp120 under physiological conditions and comprises less than about 100 amino acid residues that are identical to or substantially identical to the amino acid sequence of the human CXCR4 chemokine receptor.

IV
23. A polypeptide comprising at least a portion or all of an amino acid sequence selected from the group consisting of LVISIFYHKLQSLTDVFL (53-70), PFWAYAGIHEWVFGQVMC (85-102), EAISTVVLATQMTLGFFL (185-202),

LTMIVCYSVIKTLHAG (205-222), MAVFLLTQMPFNLMKFIRSTHW (237-258), HWEYYAMTSFHYTIMVTE (257-274), ACLNPVLYAFVSLKFRKN (281-298) and SKTFSASHNVEATSMFQL (325-342), and, optionally, substituted with up to 6 conservative or neutral amino acid substitutions, wherein the polypeptide binds with HIV gp120 under physiological conditions and comprises less than about 100 amino acid residues that are identical to or substantially identical to the amino acid sequence of the human STRL33 chemokine receptor.

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24. A polypeptide comprising at least a portion of or all of an amino acid sequence selected from the group consisting of DTYICEVED, EEVQLLVFGLTANS, D, THLLQGQSLTLTLES, and GEQVEFSFPLAFTVE, and, optionally, substituted with up to 6 conservative or neutral amino acid substitutions, wherein the polypeptide binds with HIV gp120 under physiological conditions and wherein the polypeptide comprises less than about 100 amino acids that are identical to or substantially identical to the amino acid sequence of the human CD4 cell-surface protein.

i
30. A composition comprising the polypeptide of claim 1 and a carrier.

iii
31. A nucleic acid, optionally in the form of a vector, encoding the polypeptide of claim 1, wherein said nucleic acid can be expressed in a cell and, optionally, further comprising a nucleic acid sequence that encodes a signal sequence, wherein said signal sequence is translated as a fusion protein with the polypeptide to form a signal sequence-polypeptide fusion, and wherein said signal sequence can cause secretion of at least the polypeptide out of a cell in which the nucleic acid is expressed.

i
ii
ix
x
34. A method of making an antibody, which method comprises administering an immunogenic amount of a polypeptide of claim 1 or a nucleic acid encoding the polypeptide to an animal.

XI 35. A method of inhibiting HIV infection in a mammal in need thereof, which
ii) XII method comprises administering to said mammal an effective amount of a polypeptide of
iii) XIII claim 1, a nucleic acid encoding the polypeptide or an anti-antibody to the polypeptide.

XIV 36. A method of making an antibody that binds to a gp120 envelope protein of
a human immunodeficiency virus-1 (HIV-1), said method comprising:

- (a) labeling a polypeptide of claim 1 to obtain a labeled compound,
- (b) providing a library of synthetic peptides, wherein said library consists of a multiplicity of synthetically-produced polypeptides that are homologous to a continuous region of an HIV-1 gp120 envelope protein, wherein each polypeptide of said library is substantially isolated from every other polypeptide of said library and is located in a known position,
- (c) individually contacting each polypeptide with said labeled compound such that a portion of the labeled compound can bind with the polypeptide, thereby producing a bound population of each polypeptide and an unbound population of each polypeptide,
- (d) removing substantially all of the unbound labeled compound from the position occupied by each polypeptide,
- (e) measuring the amount of labeled compound that remains co-localized with each polypeptide, to determine the quantity of labeled compound bound by each polypeptide,
- (f) evaluating the amount of labeled compound bound by each polypeptide to identify a portion of the HIV-1 gp120 envelope protein that binds to an (HIV-1)-receptor selected from the group consisting of CCR5, CXCR4, STRL33, and CD4,
- (g) providing an immunizing compound comprising a polypeptide comprising an amino acid sequence that is homologous to said portion of the HIV-1 gp120 envelope protein,
- (h) inserting an immunogenic quantity of said immunizing compound into an animal to cause said animal to produce an antibody that binds with said portion of the HIV-1 gp120 envelope protein.

50. The method of claim 36, wherein said step of removing substantially all of the unbound labeled compound comprises the additional steps of (i) removing a liquid containing said unbound labeled compound from a solid substrate to which an polypeptide of the library is bound, (ii) applying a quantity of wash-liquid to said solid substrate that is sufficient to cover any portion of said solid substrate or a vessel containing said solid substrate that has been contacted by said labeled compound, and (iii) removing said wash-liquid.

XV 53. The immunizing compound of step (g) of the method of claim 36.
3 HIV-1 gp120

XVI 54. An antibody produced by the method of claim 36.

XVII 55. A method of removing HIV from a bodily fluid of a mammal, which method comprises extra-corporeally contacting said bodily fluid with a solid support to which is attached a polypeptide of claim 1 or an anti-antibody to the polypeptide of claim 1.
ii)
ii)

XVIII 56. The polypeptide of claim 5, which consists essentially of the amino acid sequence YDIN*YYT*S*E, wherein N* is asparaginyI or a synthetic or naturally occurring substitute therefor, T* is threoninyI or a synthetic or naturally occurring substitute therefor, and S* is serinyI or a synthetic or naturally occurring substitute therefor.
I

57. The polypeptide of claim 56, wherein N* is asparaginyI, T* is threoninyI, and S* is serinyI.

II 58. A composition comprising the polypeptide of claim 11 and a carrier.

III 59. A composition comprising the polypeptide of claim 17 and a carrier.

IV 60. A composition comprising the polypeptide of claim 21 and a carrier.

5 61. A composition comprising the polypeptide of claim 22 and a carrier.

6 62. A composition comprising the polypeptide of claim 23 and a carrier.

11 63. A composition comprising the polypeptide of claim 24 and a carrier.

XIX 64. A nucleic acid, optionally in the form of a vector, encoding the polypeptide of claim 11, wherein said nucleic acid can be expressed in a cell and, optionally, further comprising a nucleic acid sequence that encodes a signal sequence, wherein said signal sequence is translated as a fusion protein with the polypeptide to form a signal sequence-polypeptide fusion, and wherein said signal sequence can cause secretion of at least the polypeptide out of a cell in which the nucleic acid is expressed.

20220313 65. A nucleic acid, optionally in the form of a vector, encoding the polypeptide of claim 17, wherein said nucleic acid can be expressed in a cell and, optionally, further comprising a nucleic acid sequence that encodes a signal sequence, wherein said signal sequence is translated as a fusion protein with the polypeptide to form a signal sequence-polypeptide fusion, and wherein said signal sequence can cause secretion of at least the polypeptide out of a cell in which the nucleic acid is expressed.

XVI 66. A nucleic acid, optionally in the form of a vector, encoding the polypeptide of claim 21, wherein said nucleic acid can be expressed in a cell and, optionally, further comprising a nucleic acid sequence that encodes a signal sequence, wherein said signal sequence is translated as a fusion protein with the polypeptide to form a signal sequence-polypeptide fusion, and wherein said signal sequence can cause secretion of at least the polypeptide out of a cell in which the nucleic acid is expressed.

XVII 67. A nucleic acid, optionally in the form of a vector, encoding the polypeptide of claim 22, wherein said nucleic acid can be expressed in a cell and, optionally, further comprising a nucleic acid sequence that encodes a signal sequence, wherein said signal sequence is translated as a fusion protein with the polypeptide to form a signal sequence-

polypeptide fusion, and wherein said signal sequence can cause secretion of at least the polypeptide out of a cell in which the nucleic acid is expressed.

44 III
68. A nucleic acid, optionally in the form of a vector, encoding the polypeptide of claim 23, wherein said nucleic acid can be expressed in a cell and, optionally, further comprising a nucleic acid sequence that encodes a signal sequence, wherein said signal sequence is translated as a fusion protein with the polypeptide to form a signal sequence-polypeptide fusion, and wherein said signal sequence can cause secretion of at least the polypeptide out of a cell in which the nucleic acid is expressed.

44 IV
69. A nucleic acid, optionally in the form of a vector, encoding the polypeptide of claim 24, wherein said nucleic acid can be expressed in a cell and, optionally, further comprising a nucleic acid sequence that encodes a signal sequence, wherein said signal sequence is translated as a fusion protein with the polypeptide to form a signal sequence-polypeptide fusion, and wherein said signal sequence can cause secretion of at least the polypeptide out of a cell in which the nucleic acid is expressed.